Optogenetic Stimulation of Dopamine Afferents in Nucleus Accumbens and Central Amygdala Reveals Differential Roles in Food and Social Motivation

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science with Honors in Biopsychology, Cognition and Neuroscience from the University of Michigan 2015

Author Note

I would like to thank Dr. Kent Berridge and Shelley Warlow for guiding me tremendously with the Honors Thesis throughout the 2014-2015 academic year. I also want to recognize Daniel Castro for suggestion on research design, Cody Schember and Josh Goldman for technical help for the research. Lastly, I want to acknowledge the Rat Resource & Research Center P40OD011062 for Th Cre Rats.

Abstract

Dopamine cells are densely located in the ventral tegmental area (VTA) and project to several forebrain structures such as the Nucleus Accumbens (NAc) and the Central Amygdala (CeA) to influence various forms of motivated actions. Previous studies have suggested that dopaminergic projections from the VTA to NAc are involved in instantaneous forms of motivation, while VTA to CeA projections may be involved in amplifying and narrowing incentive motivation to a single reward target. (Fields, Hjelmstad, Margolis, & Nicola, 2007; M. J. Robinson, Warlow, & Berridge, 2014). Here we examined both projections in the same task using optogenetic excitation of dopamine projections from VTA to either NAc or CeA to understand the differential roles that dopamine (DA) serves in influencing motivation for food and social interaction. Stimulation of DA afferents from VTA to NAc shell via optogenetic virus in Th-Cre rats caused rats to instantly bias their choice for one sucrose pellet paired with VTA to NAc stimulation over another identical sucrose pellet not paired with stimulation. Stimulation of DA afferents from VTA to CeA caused an equally intense bias as VTA to NAc, but which did not appear until the third day. Overall, rats receiving VTA to NAc DA stimulation pressed more at their laser-paired lever than those receiving VTA to CeA stimulation, earning them more sucrose pellets. Neither pathway supported self-stimulation when rats were given the opportunity to earn laser stimulation alone. In a social paradigm, rats displayed more social interactions with an unfamiliar rat when the VTA to NAc was stimulated. Our findings propose that DA afferents from VTA mediate different aspects of motivated behavior, depending on whether they project to CeA or NAc.

Keywords: dopamine, nucleus accumbens, central amygdala, reward, social play, optogenetics

Optogenetic Stimulation of Dopamine Afferents in Nucleus Accumbens and Central Amygdala Reveals Differential Roles in Food and Social Motivation

Drug addiction is a widespread societal concern due to its devastating effects on many individuals. It can be characterized by an intense pursuit of one reward over all others, often due to a dysfunction in regions of the brain that are involved in motivated behaviors (T. E. Robinson & Berridge, 2003). Another related problem is the susceptibility to relapse into addiction and intense pursuit of one's preferred drug, where relapse is thought to be due to an amplification of motivation triggered by either presence of the drug or a learned drug association (T. E. Robinson & Berridge, 2001). This heightened motivation is often exclusive to a specific reward such as drugs for a drug addict and food for a food addict, etc. Thus, an increase in motivation usually becomes focused onto a specific reward target.

To understand the biology of such aberrant motivation, it is imperative to understand the neurobiological system and circuits involved (Richard, Castro, Difeliceantonio, Robinson, & Berridge, 2013).Dopamine, a neurotransmitter densely populated in the mesocorticolimbic system, has been implicated in several motivated behaviors for natural rewards such as food, sex and drugs (T. E. Robinson & Berridge, 2003). Dopamine is released in various mesocorticolimbic structures in response to rewarding stimuli as well as stimuli predictive of various rewards (Berridge, 2007; Iversen & Iversen, 2007; Schultz, 2007). Therefore, many dopamine-based brain manipulations can significantly change incentive salience (or motivational wanting) of rewards without changing their 'liking' or even learning (Berridge, 2007). Taken together, it is likely that motivation or 'wanting' for rewards by individuals may change based on different regional brain manipulations.

Dopamine cells are densely located in the ventral tegmental area (VTA) and project to several forebrain structures to influence various forms of motivated actions. VTA dopamine neurons densely project to the nucleus accumbens (NAc) (Iversen & Iversen, 2007), and these projections are thought to be involved in several rewarding behaviors, including food seeking behaviors (Adamantidis et al., 2011) and morphine conditioned place preference (Koo et al., 2012). These VTA projections specifically to NAc have also been implicated in mediating social interactions. In particular, phasic stimulation of this pathway may modulate the effects of social defeat stress paradigms (Chaudhury et al., 2013). Several lines of research propose NAc as mediating various forms of motivation, including forming powerful associations between cues and rewards; this is likely a result of VTA dopaminergic innervation. Microinjections of amphetamine, a dopamine agonist, into NAc shell greatly increase the attribution of incentive salience to a sucrose reward cue, which triggers intense sucrose seeking in the presence of that predictive cue (Wyvell & Berridge, 2000). Similarly, mu-opioid stimulation of NAc via DAMGO is capable of amplifying cue-triggered incentive motivation (Pecina & Berridge, 2013).

In contrast, it is likely that dopaminergic projections from VTA to the Central Amygdala (CeA) induces more specific increases in motivation based on previously learned associations (Kim, Quinn, Spanswick, & O'Hare, 2009). CeA opioid enhances and focuses wanting as it recruits GABAergic neurons in CeA which may be affected by dopaminergic release from the ventral tegmental area (Kim et al., 2009; Mahler & Berridge, 2012). Specifically, microinjections of DAMGO (mu-opioid agonist) into the CeA increases appetitive behaviors (nibbles and sniffs) towards reward-related cues in a Pavlovian conditioned approach task (Mahler & Berridge, 2012). Furthermore, these enhanced appetitive behaviors were only observed in response to a cue that each rat spent time engaging with beforehand so that DAMGO enhanced goal tracking and

sign tracking behaviors but did not switch phenotypes (DiFeliceantonio & Berridge, 2012). Specific dopaminergic projections to CeA may mediate pavlovian conditioned approach, as heightened D1 receptor activation is observed following this procedure (DiFeliceantonio & Berridge, 2012). Similarly, DAMGO in CeA enhances cue-triggered 'wanting' as well as a focused desire to spend time with a female rat in estrous over a female rat not in estrous (Mahler & Berridge, 2012). Combined, this evidence supports a role for CeA in amplifying motivation, but also in focusing it on to more appropriate rewards, a contribution that differs from NAc activity.

Recent work in our lab has elucidated several distinct differences in operant behavior depending on whether central amygdala neurons or VTA dopamine neurons are optogenetically stimulated. In particular, we have found that stimulating CeA induces a strong bias for particular sucrose rewards in an operant task where rats must press a lever to earn a sucrose pellet and are given the choice between earning a sucrose pellet alone or a sucrose pellet paired with optogenetic excitation of CeA (M. J. Robinson et al., 2014). A strong bias for earning laser stimulation-paired sucrose surfaced on the third day and grew in strength over several days. Additionally, this bias persisted for up to four days (possibly more) once the laser stimulation was taken away. However, CeA excitation was not sufficient to maintain lever pressing behavior alone and an external sucrose reward was required. These findings support CeA's role in mediating a specific aiming of motivation towards one particular reward as well as the notion that CeA may be an important interface between learning of a stimulus value and appetitive and aversive motivation (Everitt & Robbins, 2005; LeDoux, 2003). Amygdala's involvement in focusing motivation for one reward above another identical one is consistent with several other studies implicating the amygdala in addictive behaviors towards drugs and drug-associated cues

(Koob & Volkow, 2010). More recently, our lab has selectively stimulated dopamine neurons in VTA via use of transgenic TH-Cre rats during a similar operant paradigm and has found certain relevant differences. In particular, VTA dopamine stimulation does not require an external reward (sucrose) to maintain behavior, and the laser-induced bias completely disappears as soon as stimulation of VTA ceases (Castro, Robinson, & Berridge, in prep).

While there are many studies that separately examine the roles of dopamine in NAc and CeA in motivated behavior, there are none that look at these two regions in contrast. Additionally, our previous studies in amygdala have optogenetically stimulated all cell bodies in these areas and have not specifically stimulated dopamine neurons. Hence, examining both projections in the same task will allow us to tease out subtle differences that could elucidate the differential roles that dopamine serves in these regions to influence motivation. Our overall hypothesis is that rats with VTA to NAc stimulation will show in-the-moment motivation alterations, while rats with VTA to CeA stimulation will show a learned motivational shift that grows over time. Several behavioral assays will be completed in order to pinpoint their different roles in operant behavior.

In addition to parsing out the roles these separate dopaminergic pathways play in operant behavior, we also examined shifts in social interactions during laser stimulation, something our lab has not previously done. Social play is a highly rewarding activity and rats are often highly motivated to play, as it also induces development of and maintains social, cognitive, and emotional processes in mammalian species (Achterberg, van Kerkhof, Damsteegt, Trezza, & Vanderschuren, 2015; Trezza, Damsteegt, Achterberg, & Vanderschuren, 2011). Dopamine has been shown by many groups to be integral to social play behavior in both humans and rodents (Northcutt & Nguyen, 2014; Plaven-Sigray et al., 2014). Indeed, CeA plays a role in processing

various forms of emotionally salient information (Gozzi et al., 2010) including the memory and generation of social interactions (Hong, Kim, & Anderson, 2014). Similarly, the nucleus accumbens shell has been widely studied in regards to social play, and seems to be preferentially involved in generation of social partner preference as well as forming associations between social play partners and odor stimuli (Paredes-Ramos et al., 2014). This evidence combined suggests that dopamine in both NAc and CeA may mediate social interaction between two rats. Our hypothesis was that stimulation of VTA to NAc will show an immediate and general increase in interactions towards rats paired with laser stimulation, while stimulation of VTA to CeA will not show instant behavioral changes, but increases in interaction may develop over days.

Here, we stimulated either dopamine neurons in VTA projecting to either NAc or CeA and gave rats the choice between earning a laser-paired sucrose pellet and a sucrose pellet alone. We then sought to examine whether rats would work harder for the laser-paired pellet using a set of progressive ratio tests. Additionally, we explored whether either of these dopaminergic pathways would support self-stimulation. Lastly, we examined how laser stimulation of the two different projections mediates social interaction between two rats.

For our study, optogenetics technique was used on Th-Cre transgenic rats. Usage of Th-Cre (rats allows us to target dopamine cells since tyrosine-hydroxylase (Th), the rate-limiting enzyme present in dopamine cells, is flagged, so that the injection of a specific viral vector infects only dopamine cells and their axons. (Lindeberg et al., 2004; Rossant, 1989; Witten et al., 2011). This is accomplished by injecting a viral vector to the desired area which will then cause expression of photoreceptors in that area's cell bodies and axons (projection sites). The optogenetics technique allows for the stimulation of respective areas by activating the viral vector, and the

temporally precise characteristics of optogenetics is useful because it allows for neuronal manipulation during specific aspects of a session, for example during only cue presentation, or one type of lever press and not another, etc. Therefore, we used optogenetics to turn specific parts of the brain on or off in order to gain or lose the function of that specific brain region (Deisseroth, 2011). Once expressed, these photoreceptors allow the neurons to be either stimulated or inhibited in the presence of certain wavelengths of light, thus delivering laser light that influences the neuronal activity (Britt & Bonci, 2013).

Method

Subjects

Nine female Th-Cre Long-Evans Hooded rats (250-350 g) of at least 3 months of age were used in this experiment. Rats were housed in a reverse 12 h light/ 12 h dark cycle (lights were on from 9pm) at 21'C constant temperature, with water and chow available ad libitum. These conditions were maintained throughout the experiment. All procedures were approved by the University of Michigan Committee on Use and Care of Animals.

Surgery

Optogenetic surgery was performed to bilaterally inject DIO-Channelrhodopsin viral vector (1μL per side) and a fiber optic implant (for eventual delivery of light). All rats were at least 9 weeks old. Rats were anesthetized with ketamine (100mg/kg, i.p.), xylazine (7mg/kg, i.p.) and atropine (0.04mg/kg, i.p.) to protect respiration before the surgery. The virus was injected bilaterally into VTA sites that project to either medial NAc shell or CeA (NAc: A/P: -5.76, M/L: ± 2.98, D/V: 8.73 20 °, Mouth: -3.3**;** CeA: A/P: -5.28, M/L: ± 3.13 20 °, D/V: -9.15, Mouth:-3.3). We injected virus (1 μl/side) for 10 minutes (0.1 μl/min), and allowed an additional 10 minutes for diffusion. Bilateral optic fibers were implanted into either medial shell of NAc (A/P:

-1.9, M/L: 3.0, D/V: 6.36 16.4 ° and Mouth:-3.3, n=2) or CeA (A/P: -1.8, M/L: ±3.7, D/V:-8.0, and Mouth:-3.3 , n=3). Rats were subcutaneously injected with antibiotic chloramphenicol (60 mg/kg, SC) and carprofen (5 mg/kg, SC) as an analgesic after the surgery. Rats were allowed five weeks for recovery and for optimal viral expression in VTA axon terminal sites.

Apparatus

All operant training and testing was carried out in operant chambers (Med Associates) controlled by Med PC software. Operant chambers consist of a clear plexiglass floor and contain two levers located on either side of a food cup where pellets are delivered, all of which are on the front wall of the chamber, and auditory speakers (for tone/white noise). Another lever is located on the back wall opposite of the food cup and served as a control lever which did not predict sucrose delivery. A video camera placed below the chamber recorded the animal's behavior at all times. During each session, lever presses and food cup entries was automatically recorded by MedPC software and Med Associates Hardware via infrared beams in the food cup and depression sensors on the levers. The operant chambers were placed inside of cabinets to reduce outside noises, distractions, and lights. The same chamber was used throughout the experiment for each rat. During self-stimulation spout tests, the same operant chambers controlled by MedPC software were used. Two empty spouts were inserted to the back wall, and a plexiglass floors were replaced by a grid floor in order to create a circuit when rats licked at either spout. In these tests, food cups were covered with metal screens. During all social interaction sessions, we used a chamber with clear plexiglass walls and floors covered with 15 cm bedding identical to bedding in the rats' home cages. Video cameras were used to record the behavior.

Procedures

Magazine training and autoshaping. The rats underwent magazine training on the first day to learn where sucrose pellets were delivered. This session consisted of delivery of 25 non-contingent pellets into the food cup, delivered roughly every 60-90 seconds. Beginning on their second day, rats underwent five days of autoshaping (also called Pavlovian Conditioned Approach). During autoshaping, there were two levers and they were located in two different locations: one to the right of the magazine and another to the left. Each lever was associated with white noise or tone. Assignment of lever location and auditory cue was counterbalanced between rats but stayed the same across days within each rat. Each session consisted of 40 trials, each trial involved extension of a lever into the chamber for 8 seconds accompanied by a tone or white noise. Once levers retracted a noncontingent sucrose pellet was delivered into the food cup.

Operant tests. Operant tests commenced five weeks following optogenetic surgery (n_{NAc}=3, n_{CeA}=6). During each operant session, rats were required to press levers in order to earn a sucrose pellet. The first four trials consisted of each lever extending into the chamber one at a time and only retracting when pressed. Once rats reached the correct ratio of lever pressing (FR1-RR4) required, that lever retracted and a sucrose pellet was delivered into the food cup accompanied by 8 seconds of tone or white noise. On one lever, laser stimulation of CeA (8 sec, 473nm, 25Hz, 3mW) also accompanied food delivery and tone. Once rats got a chance to sample each lever and its accompanying reward (4 trials, 2 lever presentations each), both levers were then extended into the chamber to allow rats to choose between them. Pressing one lever delivered sucrose accompanied by a tone (*Sucrose alone*) and pressing the other lever delivered sucrose accompanied by tone and laser stimulation of CeA (*Laser + Sucrose*; 25Hz, 3mW, 8 sec). Each session lasted thirty minutes. Rats performed Fixed Ratio 1 (FR1) for three days, in which one lever press earned one subsequent sucrose pellet. Following FR1 training, rats

performed Fixed Ratio 4 (FR4) for one day, in which four lever presses were required to earn each additional sucrose reward. Next, rats underwent RR4 session for a day where they were required to lever press roughly 4 times (on some trials 2 times and on others up to 6 times).

Estrous phase collection. Following each daily operant session, rats were vaginally swabbed in order to collect epithelial cells as a means of classifying what estrous phase rats were currently in. Our goal was to use vaginal smears to identify each rat's stage of estrous cycle (proestrus, estrus, metestrus, and diestrus) during each test day to examine any possible influence on lever preference or amount of responses. Each phase of the estrous cycle was defined by the absence, presence or proportion of certain cell types, as well as by the cell density and the arrangement (Cora, Kooistra, & Travlos, 2015). In order to collect the cells for estrous cycle, we used a pipette (new tip for each animal) and flushed 10 μL saline through the vagina opening to collect epithelial cells. Pipette contents were then placed in small drops onto labeled slides, which were analyzed using a standard microscope at both 10 and 20 times magnification.

Progressive ratio tests. Rats were given six separate progressive ratio sessions: two before they started operant training with one day being for a laser-paired sucrose pellet and another being for a sucrose alone pellet, order of days counterbalanced between animals; and the other four sessions being after operant training again with one day for earning a laser-paired sucrose pellet and another day for earning a sucrose pellet alone, with order of days also counterbalanced between animals. The progressive ratio sessions before operant training were administered to see if there are any differences between stimulated dopamine in CeA and NAc on instant motivation for a sucrose pellet. Rats performed four progressive ratio tests after completion of RR4 testing. Out of four sessions, two sessions were conducted with unlabeled levers (n_{NAc}=2, n_{CeA}=3), meaning one lever extended into the chamber and pressing it at the right

ratio delivered a sucrose pellet (accompanied by laser stimulation on laser sessions) but *not* accompanied by its previously paired tone. Thus during these sessions, levers were not labeled with any previous sensory labels such as the tone or white noise. Additionally, the lever was located in a novel location (either the near or far side of the back wall) in order to avoid any location-based sensory label from previous associations. The last two sessions (*Laser + Sucrose* and *Sucrose* alone) were labeled with their previously accompanying tone or white noise upon lever pressing (n_{NAc}=3, n_{CeA}=5). Within each progressive ratio session, the number of times rats must press the lever increases every trial at an exponential level and each session lasts thirty minutes. The following schedule of lever presses required to earn each subsequent pellet was based on Robinson et al., 2014: 1,2,4,6,9,12,15,20,25,32,40,50,62,77,95,118,145,178,219, 268,328,385, 445, 515, 585, 665, 745, 835, 925, 1025, 1125, 1235, 1345, 1465, 1585, 1646,2012,2459,3004, 3670,4484,5478,6692,8175,99999. The point at which rats stop pressing the lever in order to receive one more sucrose pellet is recorded and called the break point. The break point is the maximum effort price rats are willing to pay for an outcome, when the price progressively increases over time.

Laser extinction sessions. After rats finished their last progressive ratio session, they continued on operant testing similar to the original days at FR1 – where they had to sample each lever and its consequence twice in the beginning of the session (forced-choice) and were then given free-choice between pressing both levers throughout the rest of the session. However, this time, when pressing at the lever which had previously delivered sucrose accompanied by tone and laser stimulation (previously *Laser + Sucrose*), laser stimulation no longer occurred. Rats performed 6 consecutive daily sessions (n_{NAc}=2, n_{CeA}=3).

Sucrose extinction sessions. After laser extinction, rats underwent sucrose extinction, where lever presses on either lever did not result in any sucrose pellet delivery. However, pressing at the *Laser + Sucrose* lever still earned rats laser stimulation of CeA at the same parameters (8 sec, 25Hz, 3mW) on an FR1 ratio.

Self-stimulation spout task. A simple spout test was administered in order to give rats an opportunity to easily earn laser stimulation alone, without any externally paired reward. During the spout test, rats were given access to two empty spouts in an operant chamber; contact with one spout delivered laser stimulation (FR1, 1sec, 25Hz, 3mW) whereas contact with the other spout did nothing. Laser-paired spout location was counterbalanced between rats but stayed the same across days for each rat. There were 3 consecutive daily sessions lasting 30 minutes each. In a second set of tests, rats had access to two separate empty spouts and contacting one of them earned the same laser stimulation parameters but was now accompanied by a 1 sec tone or white noise, counterbalanced between rats. Contacting the other spout would only deliver 1 sec tone or white noise. Number of licks at each of the spouts was measured.

Social interaction sessions. Social interaction behaviors were examined over two different sessions per day, fifteen minutes each that lasted four days (n_{NAc}=2, n_{CeA}=3). Within each day, each rat was given 15 min to interact with two different ‘stranger’ rats – one which would always be paired with laser stimulation upon interaction, and another which would never be paired with laser stimulation; the order in which they interacted was counterbalanced across days and between rats. These ‘stranger’ rats had not undergone surgery and were completely novel to the experimental rats, but were weight-matched so that both rats that interacted were within 10 grams weight of each other to avoid any resident-intruder situations. During each laser session, whenever the rat approached or interacted with the new rat within 1 inch, they received

laser stimulation (25Hz, 3mW) that lasted until they stopped interacting. On the fourth day, experimental rats were given an opportunity to interact with their cage mates for a 15 minute session and to also receive laser stimulation when approaching one of those cage mates.
In each video, the following interactions were scored: Time spent ano-genital sniffing, number of pins, pounces and nuzzles, all of which have been behaviorally defined previously (Pellis and Pellis, 1987). *Ano-genital sniffing* was defined as placement of the rat's snout on or near the other rat's anus or genital region, and in order to quantify the measurement, we calculated two seconds of sniffing as one 'bout'. *Pinning* referred to one rat lying with its dorsal surface towards the floor and another rat standing over it. *Pouncing* events were counted when one rat lunged toward the back of the other (play solicitation). A *nuzzle* was defined as moving one's nose into the neck of the other rat, without biting it (Pellis & Pellis, 1987).

Viral expression. Rats were deeply anesthetized with an overdose of sodium pentobarbital and perfused. Brains were then subsequently stored in 4% paraformaldehyde, cryoprotected in 30% sucrose, and then sliced at 40m. Slices were blocked in 5% normal donkey serum/0.2% Triton-X solution for 30 min before being incubated for 24 h in a polyclonal goat anti-c-fos IgG primary antibody (Santa Cruz Biotechnology), followed 2 d later by 2 h in Alexa Fluor 594 donkey-antigoat IgG (Invitrogen) (Faure, Raynolds, Richard, & Berridge, 2008; Paxinos & Watson, 2007). Sections were mounted, air-dried, and cover slipped with ProLong Gold antifade reagent (Invitrogen). To identify fiber tip locations and assess viral spread, relevant sections were examined using a Leica microscope and results were marked on a coronal schematic in Adobe Illustrator using the rat brain atlas (Paxinos &Watson, 2007). Nine images were compiled using MCID Core 7 software 3; 10 magnification) into one single image centered on the fiber tip.

Statistical analysis. Results were analyzed using repeated-measures ANOVAs to examine the response preference for either lever, followed by t-tests for individual comparisons. For all analyses, the significance level was set at p 0.05, two-tailed.

Results

Operant Tests

We first examined how laser stimulation of VTA dopaminergic afferents in either CeA or NAc would affect choice between two identical sucrose pellets, where rats were given a choice between earning one sucrose pellet alone (*Sucrose alone*) or an identical sucrose pellet paired with optogenetic excitation of either region (*Laser + Sucrose*). Both NAc photo-excitation and CeA photo-excitation induced a significant bias for the *Laser+Sucrose* lever ($F = 28.351$, $df = 1$, $p = .001$) as well as a significant interaction between lever preference and day. Overall, rats displayed increased lever presses across days as workload increased and rats were required to lever press up to 4-6 times for each additional sucrose reward ($F = 12.316$, $df = 4$, $p < .005$). Combined lever presses at both levers grew slightly more over days in NAc rats when compared to CeA rats ($F = 2.549$, $df = 4$, $p = .06$).

On the first day, the percentage preference NAc rats had for the *Laser + Sucrose* lever was significantly greater than the percentage bias that CeA rats showed (NAc: 89.64% +/-, SE = 5.95% CeA: 66.49% +/-, SE = 7.67%; $t = 2.385$, $df = 6.735$, $p = .05$). However, there were no significant differences between lever presses at either lever in both groups of rats on the first day (NAc: $t = 1.554$, $df = 2$, $p = 0.26$; CeA: $t = 1.478$, $df = 5$, $p = .19$). NAc rats generally maintained the high bias preference for all five days while CeA rats showed a gradual narrowing of focus on the *Laser + Sucrose* lever over the five days, reaching a significant *Laser + Sucrose* lever preference on the third day (75.67% +/- SE = 7.74; $t = 2.87$, $df = 5$, $p = .035$). By the last day,

active responses on the *Laser + Sucrose* lever were significantly greater than that of the sucrose-alone lever: NAc rats reached an over 6:1 preference for the *Laser + Sucrose* lever (86.76% +/- SE = 5.78; t = 4.607, df = 2, p = .044) and CeA rats reached an over 7:1 preference for the *Laser + Sucrose* lever (t = 2.859, df = 5, p = .35) on that fifth day, reaching an 87.39% (+/- SE = 2.07) preference. While NAc rats displayed a significantly higher *Laser + Sucrose* lever preference on the first day compared to CeA rats, by the fifth and final day CeA rats caught up and displayed a similar *Laser + Sucrose* lever preference to NAc rats (t = -.105, df = 2.527, p = .924). The overall number of lever presses by NAc rats at the *Laser + Sucrose* lever reached on average 538 presses, while CeA rats reached an average of 288 *Laser + Sucrose* lever presses (t = 1.802, df = 6.001, p = .122).

Estrus Data

There was no effect of laser stimulation on estrous cycle for *Laser + Sucrose* lever compare to *Sucrose alone* lever in any of the operant schedule dates (t = -0.722, df = 12, p = .484).

Progressive Ratio Tests: Breakpoint

To asses independently whether NAc and CeA stimulation amplified motivation to work for a laser-paired sucrose reward, rats performed instrumental progressive ratio tests in which a rat faced only a single lever during each session. Our progressive ratio tests were divided into three sections: 1) pre-operant progressive ratio sessions 2) unlabeled-lever-progressive ratio sessions (no associated tone or location) and 3) labeled-lever-progressive ratio sessions (levers were the same location and had the same accompanying tone as during operant training).

Progressive ratio tests performed before operant training were used to assess whether differences might emerge in motivation before the rats have any operant and/or laser experience.

Both NAc and CeA rats showed low overall lever responses and reached low breakpoints. NAc rats pressed a total of 20 times in both laser and no-laser test days, and reached a breakpoint twice as high on the *Laser + sucrose* lever ($t = 1$, $df = 1$, $p = .5$). CeA rats pressed a total of 50 times in both laser and no-laser test day, and reached breakpoints on average 8 times higher on the *laser + sucrose lever* ($t = -1.809$, $df = 2$, $p = .199$). Thus, laser stimulation did not amplify the intensity of motivation to work for laser-paired sucrose in both NAc and CeA rats. Additionally, there was no difference between NAc or CeA pathway stimulation in breakpoints for either session ($t = -.433$, $df = 1.186$, $p = .731$).

After the operant training, we evaluated the ability of laser stimulation to amplify incentive motivation during both an set of unlabeled-lever-progressive ratio tests and a set of labeled-lever-progressive ratio tests, with rats having access to only *Laser + Sucrose* lever or *Sucrose alone* lever during each session (order of sessions counterbalanced between rats). During the unlabeled *Laser + Sucrose* lever session and the unlabeled *Sucrose alone* lever session, levers were extended into a novel location within the chamber and sucrose delivery upon meeting the ratio was *not* accompanied by its previously paired tone. During these tests, both NAc photo-excitation and CeA photo-excitation showed no increase in effort to earn sucrose. NAc rats pressed on average up to 51 times for a single laser-paired sucrose pellet and roughly 63 times for a sucrose-alone pellet ($t = -.390$, $df = 1$, $p = .76$). CeA rats pressed on average 46 times to earn a single laser-paired sucrose pellet whereas they were willing to press roughly 59 times for the sucrose-alone pellet ($t = -1.441$, $df = 2$, $p = .286$). This indicates that both NAc and CeA rats were willing to work similar amounts on both laser and non-laser sessions when the sucrose delivery was not also accompanied by a formerly paired sucrose pellet.

During the set of labeled progressive ratio tests, the *Laser + Sucrose* lever and the *Sucrose alone* levers were extended into the same location as during operant training and *were* accompanied by their previously paired tone. These tests were used to examine the effects of laser excitation on motivation in the presence of previously learned associated sensory cues, which may alter motivation on their own.

During these sets of tests, NAc rats pressed on average up to 335 times for a subsequent single laser-paired sucrose pellet and 200 times for their next sucrose alone($t = -.286$, $df = 2$, $p = .802$), earning 167% more laser-paired sucrose pellets ($t = 3.250$, $df = 2$, $p = .083$). CeA rats pressed on average up to 204 times for a single laser-paired sucrose pellet in contrast to only 147 times for sucrose alone ($t = 1.264$, $df = 4$, $p = .275$), earning 138% more pellets ($t = 1.355$, $df = 4$, $p = .247$). These results suggest rats are likely to work harder when they have previous exposure to the lever.

Furthermore, our data shows NAc stimulated rats pressed the lever significantly more on the laser day than CeA rats ($t = 2.468$, $df = 6$, $p = .049$). This indicates that NAc stimulation amplified the intensity of motivation to work for a laser-paired sucrose (accompanied by sensory cues) to a greater extent than CeA stimulation. When assessing final breakpoints reached during sensory–labeled laser stimulation sessions, NAc rats trended towards reaching higher breakpoints than CeA rats. The average breakpoint reached by NAc rats during sensory–labeled laser stimulation sessions was 69, compare to the break point reached during sensory-labeled no laser stimulation session, which was 43 ($t = 3.25$, $df = 2$, $p = .083$). The average breakpoint reached by CeA rats during sensory–labeled laser stimulation sessions was 44, compare to the break point reached during sensory-labled no laser stimulation session, which was 32 ($t = 1.355$, $df = 4$, $p = .247$).

Laser Extinction

During laser extinction, rats were presented with a choice of two levers that each earned a sucrose pellet. These levers were the same levers used in the operant tests, and pressing on each one was still accompanied by its previously paired tone. However the previous *Laser + Sucrose* lever did not provide laser stimulation in this test, resulting in both levers essentially delivering identical sucrose pellets.

Overall, both NAc and CeA rats maintained a slightly higher bias for the laser associated lever even when there was no laser stimulation presented ($F = 6.290$, $df = 1$, $p = .087$). NAc rats maintained a slightly high preference for the laser-associated lever over six days ranging from 89.24% (+/- SE = 2.26) on the first day to 79.73% (+/-SE = 10.34) on the last day ($F = 9.29$, $df= 5$, $p = .531$). CeA rats also maintained a high preference for the laser-associated lever ranging from 82.43% (+/- SE = 24.96) on the first day to 78.24% (+/- SE = 22.41) on the last day ($F = 2.435$, $df = 5$, $p = .108$). There were no differences between NAc-stimulated and CeA-stimulated rats in their preference throughout laser extinction ($F = 0.078$, $df = 1$, $p = .798$). The total number of combined lever presses dropped from on average 349 to 190 presses for NAc rats ($t = 1.053$, $df = 1$, $p = .484$) and 389 to 324 presses for CeA rats ($t = 0.908$, $df = 2$, $p = .460$), but this decline in pressing was not significantly different between the two groups of rats ($F = 1.017$, $df = 5$, $p = .442$).

Sucrose Extinction

Following laser extinction, rats were retrained for two days on the original FR1 operant conditions with both the *Laser + Sucrose* lever and the *Sucrose alone* levers to familiarize them. During sucrose extinction, they were then were presented with a choice between pressing two levers that were the same levers used in earlier operant tests. However, for this experiment, the

original *Laser + Sucrose* lever only delivered laser stimulation but no sucrose reward, and the original sucrose alone lever delivered nothing. This way, we were able to examine whether laser stimulation alone of either CeA or NAc terminals was sufficient to maintain operant behavior. Rats performed this test for three subsequent days.

Both NAc and CeA rats did not show any preference between levers when the sucrose was no longer delivered ($F = 2.682$, $df = 1$, $p = 0.243$) The average *Laser + Sucrose* lever preference dropped on the very first day to 60% and ended at 46% (+/- SE = 16.76%) by the third day with rats choosing equally between the two levers (though no sucrose was delivered at either one) ($F = 0.372$, $df = 1$, $p = .604$). Overall lever presses dropped dramatically. The total number of combined lever presses dropped from 274 to 166 presses for a NAc rat and 136 to 72.667 presses for CeA rats ($t = 2.159$, $df = 2$, $p = .164$), but this decline in pressing was not significantly different between the two groups of rats ($F = 1.017$, $df = 5$, $p = .442$).

Self-Stimulation Spout Task

In order to further investigate whether laser stimulation of either of these pathways has a rewarding effect alone, we performed a self-stimulation test. Rats were presented with two sippers: one being *Laser+ Sipper*, another being *Sipper-only*. The self-stimulation test was composed of two different sets of sessions. During the first set, rats were given the opportunity to earn laser stimulation (of either NAc or CeA afferents from VTA) (1 sec, 25Hz, 3mW) by contacting one spout and to earn nothing when contacting the other spout (control spout), over three consecutive days. The second set of sipper tests also presented two separate spouts, contact of one of these leading to laser stimulation, but each spout was also paired with a 1sec tone or white noise, pairings counterbalanced between rats. During the first set of tests (without the paired tone), both NAc and CeA rats did not show any preference for the *laser+ sipper* over the

sipper-alone (F = 0.057, df = 1, p = .827). There were also no regional differences in preference for the *laser+ sipper* (F = 1.142, df = 1, p = .364). Interestingly, there was a significant decline of total sipper contacts (both *laser+ sipper* and *sipper-alone* combined) over the three days (F = 22.742, df = 2, p = .002): from roughly 66 licks (+/- SE = 11.9) down to 15 licks (+/- SE = 5.84), and this was not different between NAc and CeA rats(F = 1.806, df = 2, p = .243).

Likewise, during the second set of tests (where activations of each spout were accompanied by a tone), both NAc and CeA rats did not show any preference for the *laser+ sipper* (F = 0.187, df = 1, p= .694). There were no regional differences in activations of *laser+ sipper* (F = .001. df = 1, p = .981). However, unlike the first session there was no significant change of total sipper contacts over three days (F = 1.013, df = 2, p = .418): from an average of 27(+/- SE = 6.8) to 28 (+/- SE = 6.8). Lastly, there were no regional differences in change in total number of sipper contact over three days (F = 404, df = 2, p = .685).

Social Play Behaviors

In order to see if the preference bias induced by laser stimulation extends to other motivated behaviors besides ingestive rewards, we conducted preliminary social tests to assess shifts in social interaction among rats when laser stimulation of either VTA pathway was administered. The following behaviors were measured and used to calculate a total social interaction score: pins, pounces, nuzzles and time spent anogenital sniffing (Pellis & Pellis, 1987). There were two different sets of tests: In the first set, both NAc rats and CeA rats were paired with two novel rats each day for 15 min each rat over three days in a row: One novel rats session involved administration of laser stimulation every time they interacted (*laser+novel rat*). On the same day but with another novel rat, there was no laser stimulation when the two rats interacted (*novel rat-only*). Order of *laser+novel rat* and *novel rat-only* sessions was

counterbalanced between days for each rat. For the second set of tests, rats were paired with their cage mates and each time they interacted, they received laser stimulation.

Although there were no significant findings, on average, NAc rats consistently spent longer time with the laser-paired rat than with the other novel rats not paired with stimulation. On average, these NAc rats interacted 374(+/- 81.5) seconds on the first day ($t = 4.595$, $df = 1$, $p = .136$), 321(+/- 103) seconds on the second day ($t = 3.117$, $df = 1$, $p = .106$), and 334.5 (+/- 75.5) seconds on the third day ($t = 4.43$, $df = 1$, $p = .141$). On the other hand, CeA rats showed on average of 175 (+/- 40) seconds on the first day ($t = 4.380$, $df = 2$, $p = .141$), 172.6 (+/- 23.1) on the second day ($t = 7.462$, $df = 2$, $p = .017$), and 280.6(+/- 49.72) seconds on the third day ($t = 5.645$, $df = 2$, $p = .030$).

When interacting with novel rats, there was a slight increase in total numbers of social interaction behaviors observed in both groups during the *laser+novel rat* sessions when compared to the *novel rat-only* sessions ($F = 5.454$, $df = 1$, $p = .101$). Between groups, NAc displayed almost significantly higher numbers of total interactions than CeA rats ($n = 5$, $F = 5.698$, $df = 1$, $p = .097$). The total number of social interactions of NAc rats across all three days during *laser+novel rat* sessions was 82.5 (+/- SE = 33) and during *novel rat-only* sessions was 87.5 (+/- SE = 22.5) ($t = -0.455$, $df = 1$, $p = .728$) while the total number of social interactions of CeA rats in all three days during *laser+novel rat* sessions was 30.3 (+/- SE = 11.5) and during *novel rat-only* sessions was 49 (+/- SE=19.76) ($t = -0.607$, $df = 1$, $p = .606$).These behaviors include ano-genital sniffing, and aggressive social behaviors such as pins, pounces and nuzzles increased noticeably when they were laser stimulated during the interactions. The Table 1 outlines each social behavior demonstrated in each session.

When NAc rats were presented with their own cage mate, the number of interaction dropped to equal levels between the two rats ($t = 3.6$, $df = 1$, $p = .172$). Similarly, when CeA rats were presented with cage mate, their duration and total number of interactions also dropped to equal levels between the two rats($t = 1.913$, $df = 2$, $p = .196$).

Discussion

Here, we used optogenetics to specifically stimulate dopamine cells from ventral tegmental area to either nucleus accumbens shell or to central nucleus of amygdala to examine the differential roles these pathways play in food and social motivation. This study supports our hypothesis, which is that NAc stimulation generally increases motivation, while CeA stimulation narrows the focus of motivation toward the reward associated with the laser stimulation over time. These unique roles were identified with both sucrose rewards and during social interactions.

In our experiment, the rats were able to control the amount of reward they could receive through lever pressing. They were presented with two levers (*laser+sucrose lever* and sucrose-alone lever), and the effort required to earn a sucrose reward increased as tests day progressed. Optogenetics (ChR2) stimulation of both the NAc and the CeA focused and narrowed the intensity of incentive motivation for that reward over days. However, subtle differences between the two pathways emerged immediately on that first day of operant training. In the case of NAc-stimulated rats, the change in preference bias was relatively instantaneous from the beginning, while in CeA the laser preference was gradual, but caught up to that of NAc rats during the last day. This result replicates the findings of other studies in our lab: VTA stimulation promotes a general reward-seeking circuit (Castro, Robinson, & Berridge, in prep). Additionally, the narrowing of focus toward laser-associated lever for the sucrose on CeA rats is consistent with

past research from our lab (Robinson, Warlow, & Berridge, 2014). This suggests that the afferent modulation by VTA dopamine in both NAc and CeA may be responsible for the findings from these previous experiments.

The mainstream view has consistently supported the theory that NAc is known to mediate motivated behaviors (Paredes-Ramos et al., 2014; Salamone, Cousins, & Bucher, 1994; Zhang, Balmadrid, & Kelley, 2003). The dopamine system originating in VTA is known to drive motivation and to assign incentive salience (Basar et al., 2010; Berridge, 2007). Dopamine in NAc is known to mediate the primary reinforcing effects of rewards as it mediates a motivated response to conditioned stimuli (Salamone, Correa, Mingote, & Weber, 2003). This possibly occurs due to dopamine's effect at either D1 or D2 receptors in NAc shell, which may in turn promote alterations in receptor expression. Specifically, an increase in dopamine D2 receptor expression in NAc has been associated with motivation for operant responding as work requirement increases, and for the reward that is less preferred but easier to access (Trifilieff et al., 2013), an effect that is shown here in this study. Likewise, our study, which confirms that NAc stimulation of dopamine cell instantly increases the laser preference.

On the other hand, the amygdala has been historically known as the center of emotion processing that is related to learning. Nevertheless, recent findings on CeA suggest this dominant view is not always the case and that the amygdala may serve as an interface of several functions. Unlike basolateral amygdala (BLA), which is part of an internal circuitry that does the traditionally known function of encoding emotional events, CeA, which is a part of the striatal circuitry, mainly serves to reinforce the motivation for an affective reward (eg. 'wanting') (Balleine & Killcross, 2006). Our study further supports CeA's role as an interface of learning and motivation. Unlike NAc rats' instant laser preference bias, CeA rats showed a gradual

change in bias for the lever preference: their laser-paired lever preference did not occur until the third day of operant testing. This may imply that CeA does not generally increase motivation but rather narrows the focus of motivation and pursuit of rewards based on some learned association. Interestingly, preference bias began on the third day of operant test in CeA rats and was a consistent trend throughout the study. This pattern had been discovered in previous studies in our lab, both with sucrose and cocaine reward (Robinson, Warlow, & Berridge, 2014; Warlow, Robinson, & Berridge, 2015, in prep).

To understand what is so “magical” about the third day, further studies can be done to understand the associative learning that might be happening from the second day to third day. Indeed, the intense bias may have been recruiting PKA-dependent synaptic plasticity in the amygdala as this form of plasticity has shown to increase in reward related learning when the cAMP pathway is activated (Olausson, Jentsch, & Taylor, 2004). Similarly, an increase in PKA activity within the amygdala facilitates appetitive-learning-associative motivation. This appetitive motivation possibly increases the potential motivational significance of incentive stimuli (Olausson et al., 2004). Also, PKA-induced synaptic plasticity required a certain amount of time to stabilize, as the infusion kicks in after a day. Hence, this may explain major increase from day two to day three in CeA-stimulated rats during our study.

Although the difference in behavior is apparent upon stimulation of different regions, the mechanism is still unclear. Therefore, a more precise targeting of receptors within each target region (NAc or CeA) may uncover how dopamine is engaging these forebrain structures to generate such intense motivation.

Similarly, the mechanism by which CeA focuses motivation is still debated. It has been proposed that CeA is an extension of striatum (Swanson, 2003). The striatum projects

motivational information to autonomic and motor nuclei (Badrinarayan, Prater, & Orsini, 2012). Therefore, if CeA is related to striatum, its role will be more closely linked to directing the behavior for the reward rather than giving a general motivational value for the reward. If CeA is only encoding general motivational value, then we should have seen both an instant increase in motivation and an increase in total number of lever presses. However compared to NAc rats, CeA rats' increase in bias was a gradual change; additionally, the total lever presses were considerably less than that of NAc rats. Yet, CeA still plays a role in generating a form of motivation, specifically a gradual narrowing towards a laser associated reward, rather than initiating heighten motivation for that reward (Swanson, 2003). Therefore, our findings in both the operant test and the failure for CeA rats to self-stimulate during the spout test align with the idea that CeA is an interface of several functions that both encodes motivational value and mediates the behavioral output. However, future studies will more precisely parse out CeA's modulation of motivational value alone versus behavioral output.

Our initial operant tests were not sufficient to conclude whether the increase in responding over days was due to laser stimulation of those pathways or to increase in effort required by each rat. It will be important in the future to test some rats with laser stimulation while keeping the work-load stable over days. To observe any further differences in incentive motivation in this study, we used a separate progressive ratio test and found no differences in breakpoints between laser-associated lever and no laser-associated lever across all three sets of tests. Interestingly, higher breakpoints were observed overall when the lever was labeled with its previously paired tone and location. It is possible that the tone could be serving as a conditioned reinforcer because with repeated sessions, the rats associated their tone with sucrose which began to acquire value on its own and gain incentive salience. This shows that photo-excitation cannot

merely hijack a choice, but in order for the amplification of motivation to happen, previous learned experience may be needed. Novel tones and/or cues in future studies may be helpful in testing whether this rats actually require a specific, previously-learned associative tones or cues to induce motivation.

The failure for laser stimulation to increase breakpoints is not consistent with previous work in our lab. However, these studies stimulated the entire VTA cells or the entire CeA neuronal population whereas our experiment activated only dopamine from VTA to CeA or VTA to NAc. Therefore, our result may indicate that stimulation of just one of these pathways is not sufficient to cause an intense increase in motivation, as measured by a break point, and may not be generated by dopamine alone. Dopaminergic neurons indeed code for magnitude of motivation and are involved in a significant biasing effect in action selection (Basar et al., 2010). Our results here suggest that dopamine release from VTA to NAC is sufficient to create a biasing effect, but not for an increase in motivation. Not only does NAc receive an immense dopamine innervation from VTA, but other areas also receive dopamine from VTA (Basar et al., 2010). Perhaps, these other pathways must also be stimulated to increase motivation.

In our laser extinction test, we expected NAc rats to show a rapid decrease in preference bias, while CeA rats would maintain their bias for a period of time. However, our results indicated both NAc and CeA maintained preference for the laser-associated lever even though there was no presence of laser. The CeA rats showed consistent result from previous study done by Robinson, Warlow and Berridge, but did not support our hypothesis regarding NAc rats. This may imply that both pathways are involved in using learned outcomes to guide future behaviors.

The sipper test results are consistent with our lab's previous experiments for CeA rats, but not NAc rats. In our previous study on VTA, we optogenetically activated the entire regions,

while in this study we stimulated only the VTA to NAc projection. Therefore, we can conclude that the self-stimulation in previous research was supported by multiple dopaminergic pathways. Other studies also seem to suggest that NAc-activated self-stimulation may be path specific. For example, activation of BLA to NAc pathway supports self-stimulation, but the optical stimulation of the mPFC to NAC pathway failed to show self-stimulation in rats, though its conjunction with dopamine signaling in NAc promotes motivated behavior responses through the glutamatergic dependent pathway, which is composed of excitatory synaptic responses from medial prefrontal cortex to the NAc (Stuber et al., 2011). This not only means that self-stimulation is pathway-specific, but also dopamine may not be enough, and requires integrative roles of other types of neurotransmitters.

In order to assess whether these pathways mediate only certain rewards but rather other types of motivated behaviors, we conducted preliminary studies to test social interactions between rats. NAc rats showed a noticeable increase in social behaviors when they received laser stimulation while interacting with novel rats, whereas CeA rats did not show much difference. The amount of pinning, pouncing, nuzzling and ano-genital sniffing generally increased when NAC was stimulated, but not notably in CeA rats over all three days. Indeed, it is possible that an alteration of NAc pathway induces behavioral changes. Dopaminergic inputs from VTA to NAc have been suggested as one of the key pathways regarding motivation especially in social interaction (Nestler & Carlezon, 2006). It has been identified as a pathway that is involved in mediating acute response to natural rewards, and failure to do so can create behavioral abnormalities such as depression and other mood disorders (Kelley & Berridge, 2002; Nestler & Carlezon, 2006). Although other studies have failed to show that altering NAc regions may induce change in social behaviors, this may be due to the use of very general dopamine and

norepinephrine reuptake inhibitors (Achterberg et al., 2015). Therefore, our results suggest perhaps it is the dopamine in NAc that modulate a change in social behavior. Moreover, our findings are consistent with a past study, which showed that stimulation of opioid receptors in the NAc increases social play such as pinning and pouncing (Trezza et al., 2011). The larger body of literature continues to support the idea that mu-opioid receptor stimulation increases positive social behavior. In this current study, we are able to go one step further suggesting dopaminergic neuron's integrative role in this trend of social behaviors. Mu-opioid receptors and NAc dopamine may work together and increase motivation in both reward seeking and social interactions (Guy, Choi, & Pratt, 2011).

On the other hand, CeA stimulation did not induce behavioral changes as it is assumed to play a role in narrowing the focus. In our operant test, the CeA rats were given a choice. Therefore, they were able to narrow the focus by having preference for one lever over the other. However, in our social interaction test, CeA rats did not have an option to choose within each session. As a result, without a "choice" to make, it is possible the bias in behavior did not occur. A further study can be conducted in order to quantify the differences with a base line. A past study from our lab showed that mu-opioid stimulation of CeA induced male rats' motivation for approaching hormonally induced-to-estrus estrous rats when they were presented with two female rats and were given a choice to approach either non-estrous rats or estrous rats (Mahler & Berridge, 2012). In a new experiment, rats could be presented with both laser paired novel rats and non-laser paired novel rats simultaneously to see if there is a change in motivation and change in preference bias.

It is also possible that the result could have been different if the duration of our testing days was longer. As mentioned earlier, CeA rats tend to show more of a snowball effect

represented by a significant narrowing of focus after the third day. Therefore, in a new experiment with a longer testing duration, perhaps five to seven days, CeA rats may show significant differences in social behavior between laser stimulation and no laser stimulation. This could also further support the serial model of the amygdala: its role as an interface of several functions as BLA encodes emotionally driven motivation, while CeA mainly serves to reinforce learning-associated motivation (Achterberg et al., 2015).

The decrease of social behavior when an optogenetics rat interacts with a cagemate may show that the effect of stimulation only applies to "reward" and not neutral stimuli. A majority of studies use single-housed rats found that the presence of another rat can be rewarding (Pellis, 1987). Since our rats were housed with their siblings, the rewarding effect is limited because they are habituated to cage mates. Another possibility is that this could be due to the ordering bias. In our study there was no counterbalance in order between novel-rats-sessions and cagemate-sessions. Therefore, further research is necessary to determine whether the effect of laser stimulation on the increase in pro-social behavior only occurs during novel rat encounters. Overall, our experiment had few limitations. One of the biggest limitations in our study was that it was conducted with only a small number of subjects. Although the trends in our results were noticeable and significant, the statistical power was not usually strong enough to make a conclusion. Further studies with larger subjects are recommended. This would potentially eliminate low statistical power and further validate the results from this study.

Another limitation is the absence of control virus animals. One can possibly suggest that our operant test results were actually influenced by the laser light in the brain, rather than the virus. Possible alternate hypothesis may be that the light from the laser could be an extra sensory

cue or that the photo excitation may heat up the surrounding brain region. Hence, further studies with control virus rats will help answer the question.

Another confounding variable in the social interaction experiments may be the differences in the "mate's responsivity." For example, certain novel rats were highly active and initiated more social activities toward the NAc and CeA rats. Since this was our preliminary study, this was not something we were aware of when we first designed this experiment. We made sure each pair would have similarity in their age and weight. However, in future experiments, precise control for the mate's selection for how reactive they are may be encouraged to reduce any further unintended bias.

Lastly, a recently published paper suggested that Th Cre mice identified non-dopamine cell specific patterns, which could limit cell-type-specific experiments (Lammel et al., 2015). Therefore, further studies are recommended to confirm our experimental results were dopamine cell are more confidently targeted.

Conclusion

Our study confirms that NAc and CeA play different roles and alter the intensity of incentive motivation. NAc stimulation instantaneously increases motivation, while CeA stimulation narrows the focus of motivation toward the reward that is associated with the laser stimulation. Our results show VTA to NAc and VTA to CeA pathways play a role inducing preference for one reward over the other, but may not be sufficient when stimulate alone to increase motivation for the reward itself.

Our study offers a possible explanation as to why many individuals may suffer from addiction to several things at once, for example, drugs and alcohol, but not all drug addicts or

alcoholics. Furthermore, addicts are not generally addicted to every possible reward; not every individual addicted to drugs is addicted to sex or gambling, and vice versa. This may be due to the fact that acquisition of reward that heightens motivation is limited to the specific reward through learned-associative narrowing of focus.

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Tables

Table 1

Social Interactions									
		Nac				CeA			
	Behavior	laser	no laser	$t_{df=1}$	p	laser	no laser	$t_{df=2}$	p
Day1	pins	**10.5** (+/- SE=1.5)	**0** (+/- SE=0)	7	0.09	**5.3** (+/- SE=5.3)	**0.3** (+/- SE=0.3)	0.908	0.46
	pounces	**12** (+/- SE=7)	**3.5** (+/- SE=2.5)	1.889	0.31	**1.6** (+/- SE=1.2)	**4** (+/- SE=2.08)	-0.803	0.506
	nuzzles	**9.5** (+/- SE=0.5)	**0.5** (+/- SE=0.5)	3	0.205	**3.3** (+/- SE=2.02)	**1.3** (+/- SE=1.3)	0.795	0.51
	anogen sniff	**45** (+/- SE=23)	**25** (+/- SE=22)	20	0.032	**18** (+/- SE=5.86)	**16** (+/- SE=11.8)	0.212	0.852
	total			6.294	0.1			0.415	0.719
Day2	pins	**4.5** (+/- SE= 4.5)	**0** (+/- SE=0)	1	0.5	**1** (+/- SE=1)	**3** (+/- SE=5.5)	-0.655	0.58
	pounces	**8** (+/- SE=8)	**2** (+/- SE=2)	1	0.5	**3.7** (+/- SE=2.3)	**7.3** (+/- SE=3.7)	-1.571	0.257
	nuzzles	**0** (+/- SE=0)	**1.5** (+/- SE=1.5)	-1	0.5	**1.7** (+/- SE=1.2)	**2** (+/- SE=1)	-0.378	0.742
	anogen sniff	**37** (+/- SE= 11)	**30** (+/- SE=4)	1	0.5	**4** (+/- SE=3)	**25** (+/- SE=11.6)	-1.883	0.2
	total			-1.16	0.451			-1.44,	0.286
Day3	pins	**11** (+/- SE=9)	**2.5** (+/- SE=1.5)	0.81	0.567	**1.7** (+/- SE=1.7)	**0** (+/- SE=0)	1	0.42.
	pounces	**10.5** (+/- SE=0.5)	**2** (+/- SE=0)	17	0.037	**10.3** (+/- SE=5.3)	**4.3** (+/- SE=1.85)	1.708	0.23
	nuzzles	**9** (+/- SE=1)	**1.5** (+/- SE=0.5)	5	0.126	**1** (+/- SE=0.57)	**2** (+/- SE=1)	-1.732	0.225
	anogen sniff	**53** (+/- SE=23)	**12** (+/- SE=1)	2.727	0.224	**30.3** (+/- SE=12.25)	**28.66** (+/- SE=1.5)	0.109	0.923
	total			2.49	.243			0.655	.580

Note: Following data are the average number of social interactions behaved by both NAc rats (n=2) and CeA rats (n=3) during three days of *laser+novel rat* sessions and *novel rat-only* sessions. NAc rats significantly performed more anogenital sniff on the first day and pounces on the third day during *laser+novel rat* sessions.

Figures

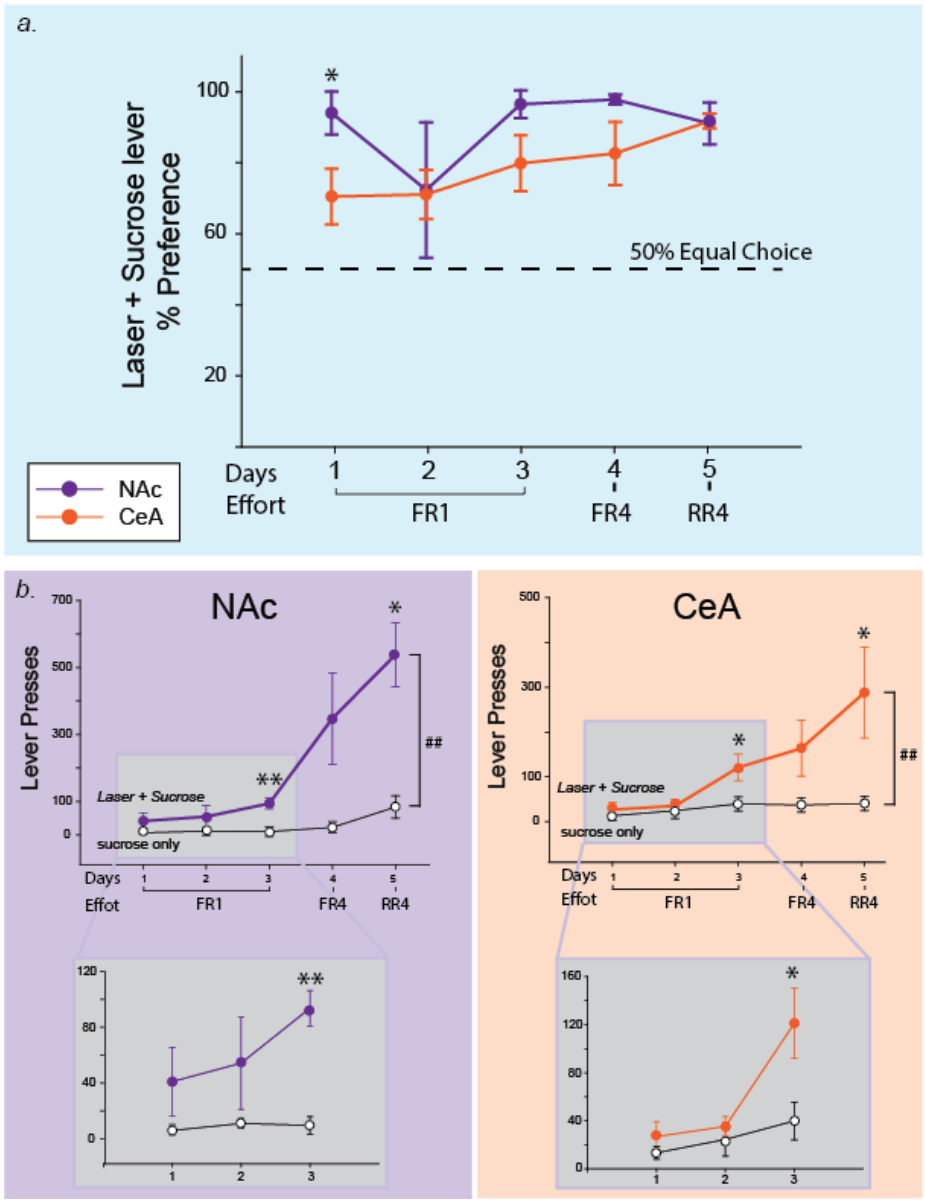

Figure1. Optogenetic stimulation of the NAc causes an instant bias for *Laser + Sucrose* lever while CeA gradually focuses choice onto *Laser + Sucrose* lever. A strong preference developed across five days of training with increasing effort requirements (FR1→ RR4) *a.* VTA to NAc pathway (n=3) stimulation displayed a significantly higher *Laser + Sucrose* lever preference on the first day compared to VTA to CeA pathway (n=6), by the fifth and final day CeA rats caught up and displayed similar *Laser + Sucrose* lever preferences with NAc rats. *b.* NAc rats show higher lever presses instantaneously compare to that of CeA rats for *Laser + Sucrose* lever. In VTA to CeA pathway, the bias surfaced on the third day and grew in strength over several days. Data are shown as mean ±SE. *p < .05; **p < .01 for independent t-test, ## p< 0.05 repeated-measures ANOVA.

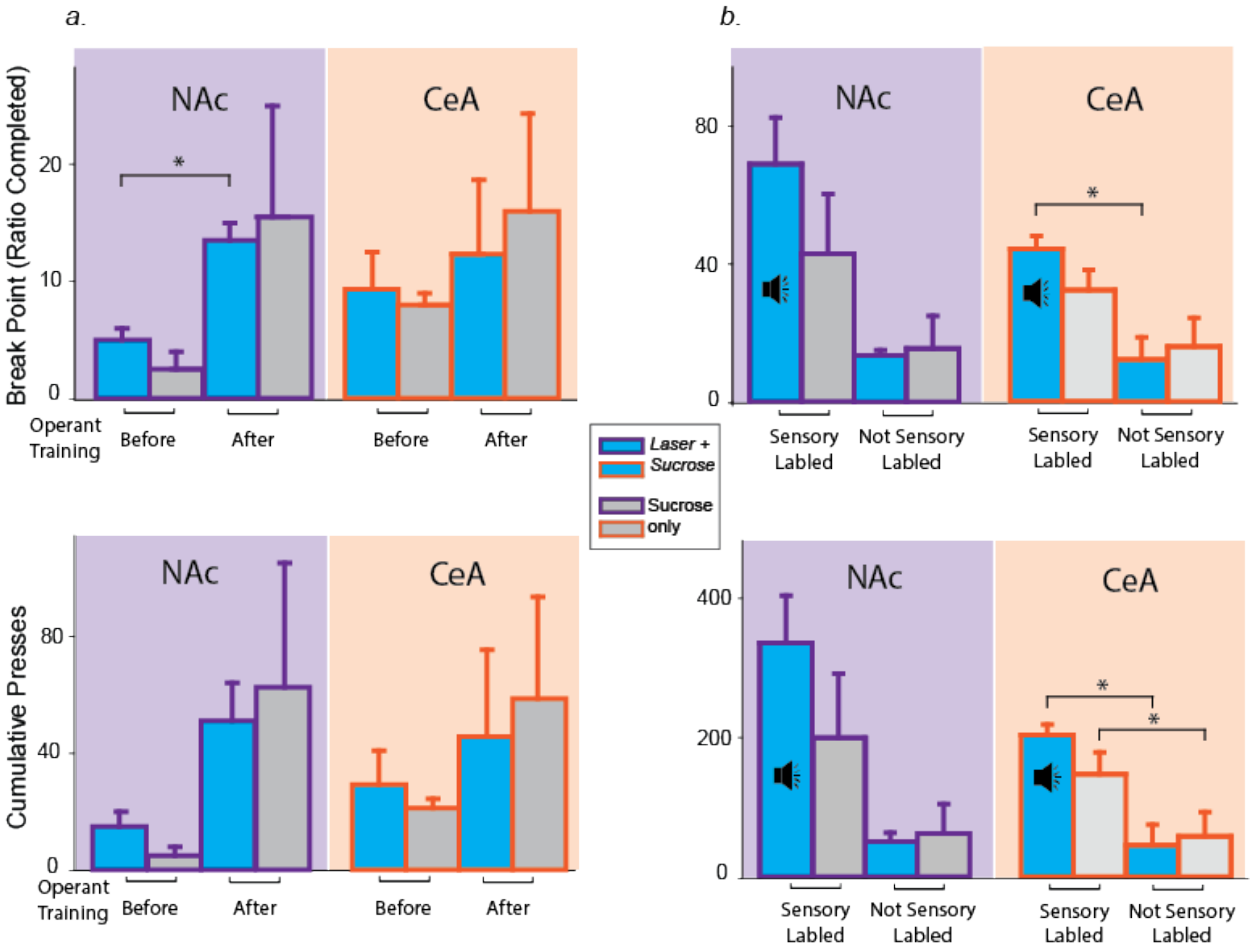

Figure 2. No Breakpoint enhancement of motivation intensity. Neither VTA to NAc nor VTA to CeA ChR2 increased breakpoint and make rats work harder to earn sucrose. Laser stimulation of NAc or CeA failed to make rats press more as effort requirement increased throughout the session in both unlabeled-lever-progressive ratio test (no associated tone or location) and labeled-lever-progressive ratio test (levers were the same location and had the same accompanying tone as during operant training). *a.* Both NAc and CeA rats failed to show a significant amplification of motivation with an unlabeled lever. *b.* When a lever is labeled, both NAc and CeA rats were willing to work harder to receive one *Laser + Sucrose* pellet. Data are shown as mean ±SE. *p < .05.

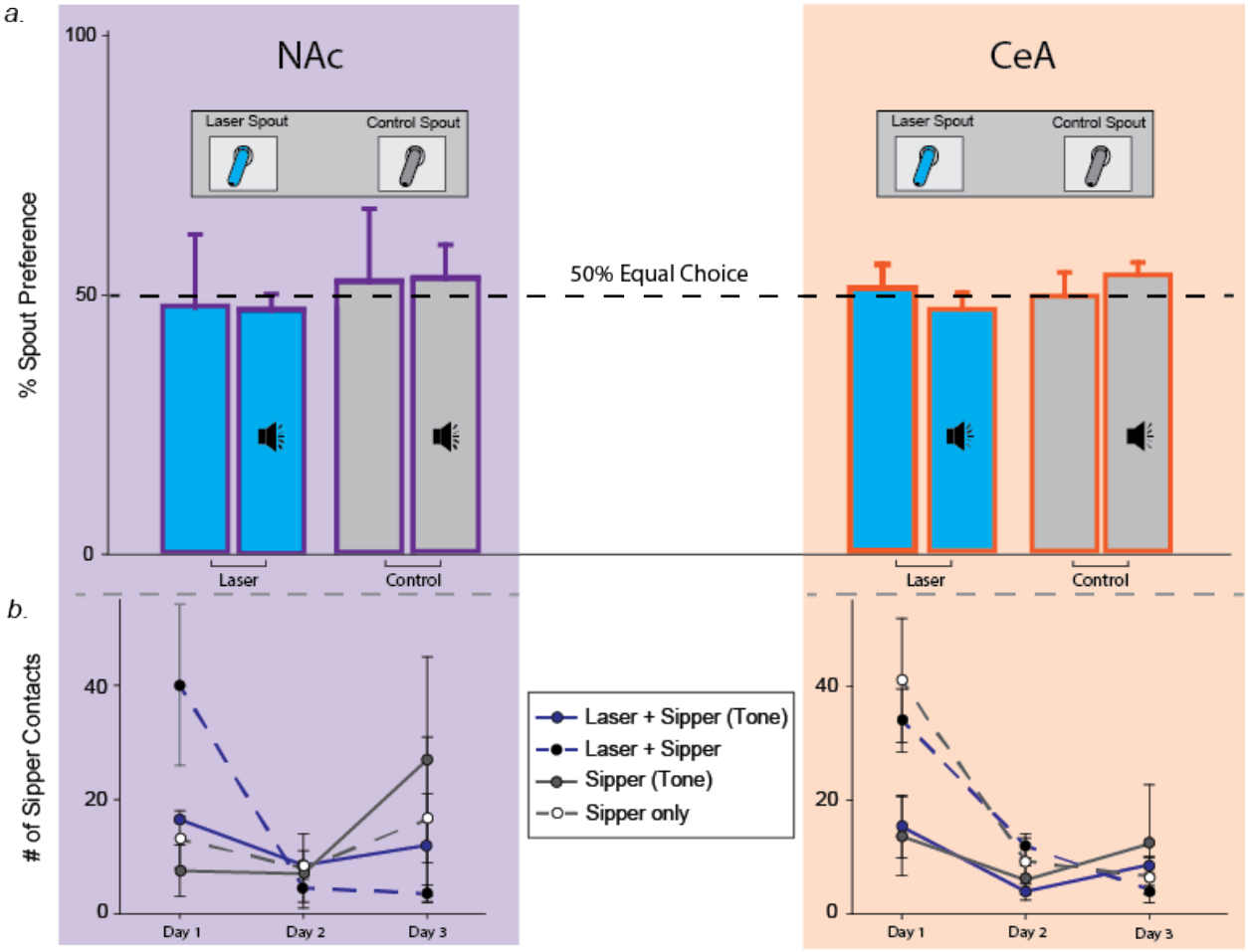

Figure 3. No self-stimulation for ChR2 laser by itself for both NAc (n=2) and CeA (n=3). In a spout-touch self-administration test, rats did not touch the empty metal spout that gives ChR2 excitation to the respective region. *a.* Both NAc and CeA rats did not show any preference for the *Laser+ Sipper* over the sipper-alone. Addition of an accompanying tone does not induce motivation. *b.* The number of sipper contacts decreased over three days. Data are shown as mean ±SE.

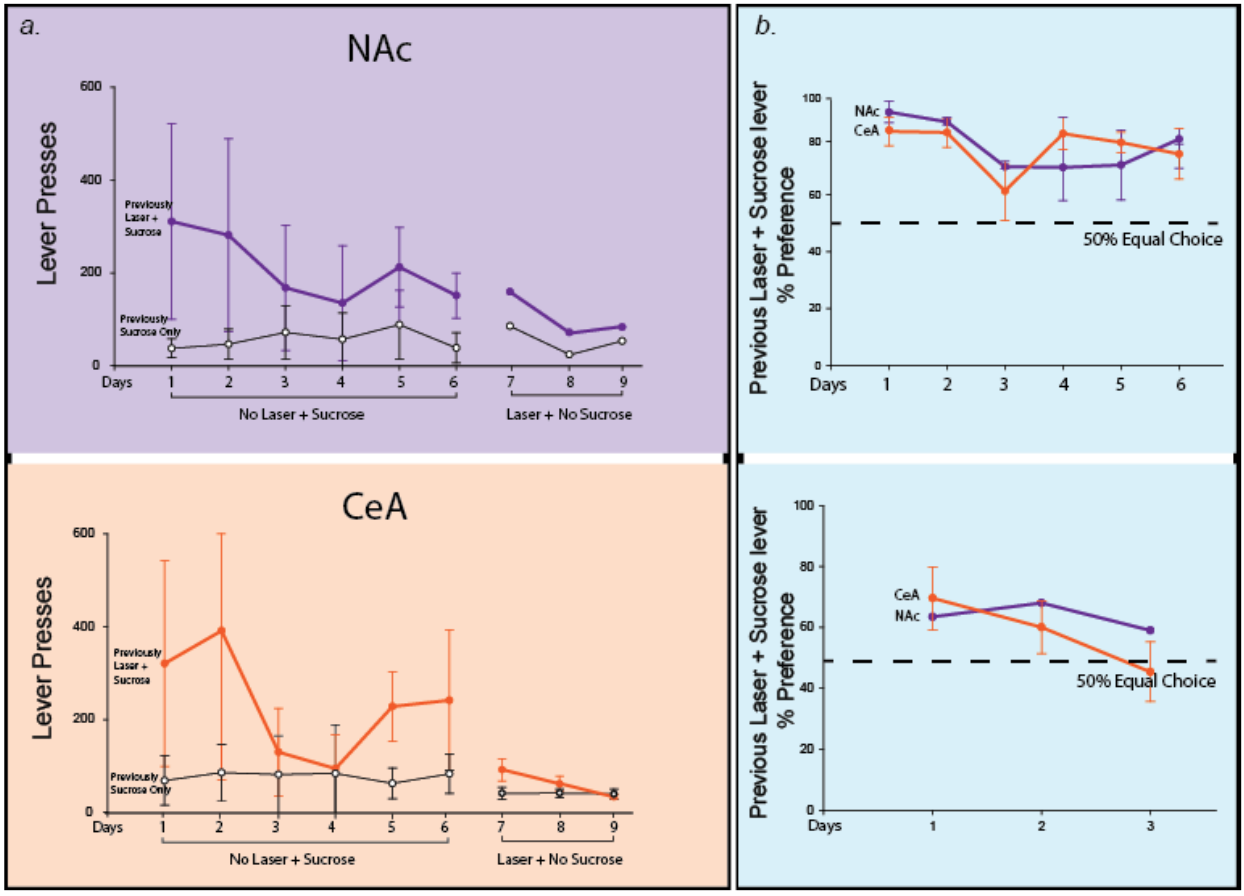

Figure 4. a. Both VTA to NAc and VTA to CeA pathways demonstrated a decrease in lever presses over nine days of laser extinction (six days) and sucrose extinction (three days). *b.* Both VTA to NAc (n=2) and VTA to CeA (n=3) pathway maintained percentage preference bias for previously *Laser + Sucrose* lever even though ChR2 laser was not presented over six days. However, both VTA to NAc (n=1) and VTA to CeA (n=3) rats demonstrated a decrease in percentage preference bias for previously *Laser + Sucrose* lever when sucrose reward was not presented over three days. Data are shown as mean ±SE.

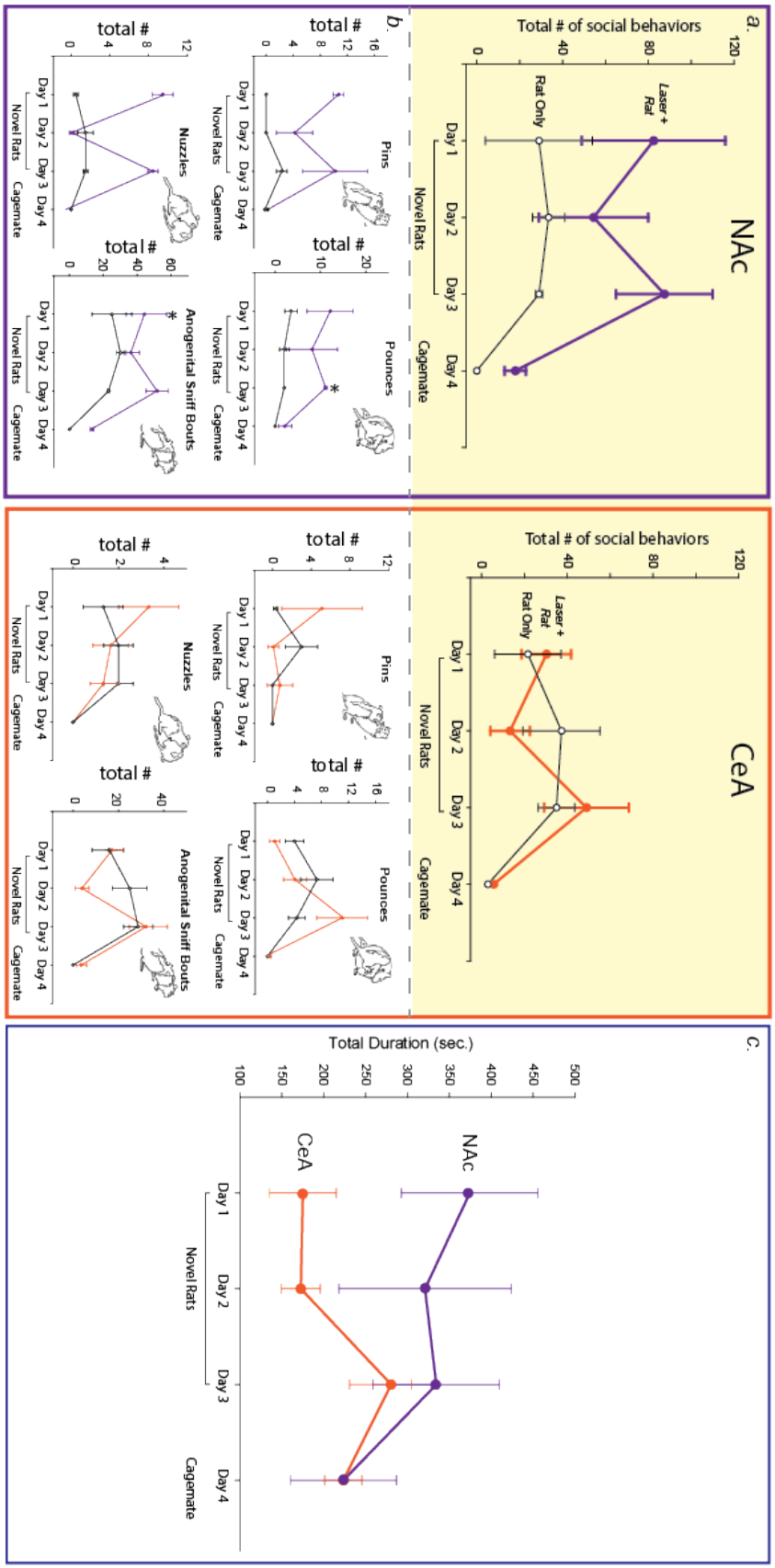

Figure 5. a. Optogenetic stimulation of VTA to NAc pathway (n=2) increased total number of social interactions during fifteen minutes *Laser + Rat* session compare to fifteen minutes Rat only session. However, VTA to CeA pathway (n=3) did not induce increase in social behavior. *b.* VTA to NAc stimulation generally increased the number of all measured social behaviors: pins, pounces, nuzzles and anogenital sniff bouts. VTA to CeA stimulation did not increased the number of measured social behaviors except pounces. During a cage mate session, ChR2 laser stimulation induced little to no social behaviors. c. NAc rats consistently demonstrated a longer time spent interacting during *Laser + Rat* session compare to that of CeA rats over three days. Data are shown as mean ±SE. *p < .05.

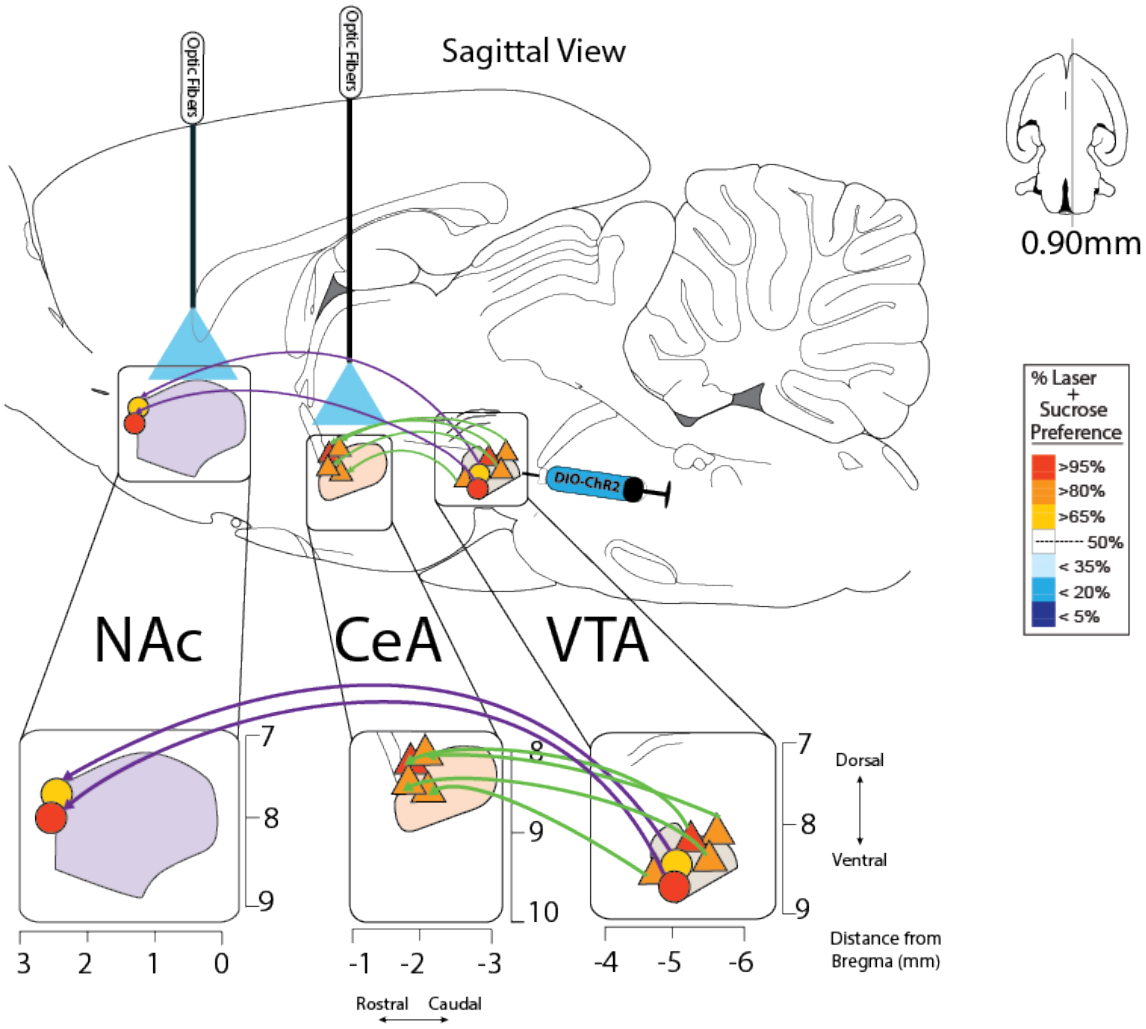

Figure 6. Localization of function maps for incentive preference bias. Maps show sites in VTA to NAc and VTA to CeA projection corresponding to data in Figure 1 for ChR2 enhancement in preference bias of sucrose choice. Color of each symbol in map represents the behavioral consequence of ChR2 laser stimulation at that site in the operant test (% laser preference for the *Laser + Sucrose* lever). The circles and arrows depict VTA to NAc pathway (n=3) and triangles and arrows depict VTA to CeA pathway (n=5). DIO-Channelrhodopsin viral vector was injected into VTA sites and optic fibers were injected into either NAc or CeA sites.